

## AMENDMENTS TO THE SPECIFICATION

Please amend the third paragraph on page 14 through the first line on page 16 of the specification as follows:

### EXAMPLE 2 – Treatment of Pulmonary Hypertension with a Survival Factor, VEGF

The full-length coding sequence of VEGF<sub>165</sub> was generated by reverse transcription-polymerase chain reaction (RT-PCR) using total RNA extracted from human aortic smooth muscle cells and the following sequence-specific primers: sense, 5'-TCGGGCCTCCGAAACCATGA-3' (SEQ. ID. NO. 1); anti-sense, 5'CCTGGTGAGAGATCTGGTTC-3' (SEQ. ID. NO. 2). This generated a 649-bp fragment that was sequenced and cloned into the expression vector pcDNA3.1 (Invitrogen) at the *EcoRI* restriction site, and correct orientation was determined by use of a differential digest. The insert-deficient vector (pcDNA3.1) was used as a control for the MCT experiments. Smooth muscle cells were transfected by use of Superfect (Qiagen Inc) with either pcDNA3.1 or pVEGF and were then trypsinized and divided into aliquots of 500 000 cells. Six-to 8-week-old Fisher 344 rats were injected with saline to establish normal hemodynamic and morphometric parameters (n=7). Experimental animals were injected with 80 mg/kg MCT SC (Aldrich Chemical Co) either alone (n=9) or together with 500,000 pVeGF – (n=11) or pcDNA 3.1-(n=10) transfected cells delivered via a catheter in the external jugular vein. At 28 days after injection, the animals were reanesthetized, and RV systolic pressure (RVSP) and systemic arterial pressure (SAP) were recorded in a Millar microtip catheter inserted into the RV ascending aorta. Before the catheter was placed into the aorta, 0.5 mL of arterial blood was drawn into a heparinized syringe and immediately analyzed for pH, PCO<sub>2</sub>, PO<sub>2</sub>, and oxygen saturation with a blood gas analyzer. The animals were then killed and the hearts excised. The RV to left ventricular (LV) plus septal weight ratios (RV/LV ratio) were determined as an indicator of hypertrophic response to long-standing pulmonary hypertension. L lungs were flushed via the pulmonary artery and gently, insufflated with 2% paraformaldehyde via the trachea. The RV systolic pressures and RV/LV ratios were compared between the pVEGF, pcDNA 3.1, and MCT-alone groups.

To determine the effect of cell-based gene transfer of VEGF<sub>165</sub> on established pulmonary hypertension, 6- to 8-week-old Fisher 344 rats were injected subcutaneously with 80

mg/kg of MCT. Fourteen days after MCT injection, the animals were anesthetized, a Millar catheter was passed into the RV, and the RV pressure was recorded. Pulmonary artery smooth muscle cells transfected with either pVEGF (n=10) or pcDNA 3.1 (n=8) were then injected in aliquots of 500,000 cells into the external jugular vein, and the animals were allowed to recover. At 28 days after MCT injection (14 days after gene transfer), the animals were reanesthetized, and RVSP, SAP, and RV/LV ratios were determined as described above.

RNA extracted from rat lungs was quantified, 5 µg of total RNA from each animal was reverse-transcribed, and an aliquot of the resulting cDNA was amplified by PCR using the following sequence-specific primers: sense,

5'-CGCTACTGGCTTATCGAAATTAATACGACTCAC-3' (SEQ. ID. NO. 3); antisense,

5'-GGCCTTGGTGAGGTTTGATCCGCATAAT-3' (SEQ. ID. NO. 4), for 30 cycles with an annealing temperature of 65°C. The upstream primer was located within the T7 priming site of the pcDNA 3.1 vector, and the downstream primer was located within exon 4 of the coding region of VEGF, thus selectively amplifying a 480-bp fragment only in the presence of exogenous pVEGF mRNA. To amplify the total cellular VEGF transcript, i.e., both exogenous and endogenous, a second aliquot of the same reverse-transcription reaction was amplified with the following primers: sense (located within the 5' UTR of the VEGF transcript), 5'-

TCGGGCCTCCGAAACCATGA-3' (SEQ. ID. NO. 5) ; anti-sense (located within exon 8), 5'-CCGCCTCGGCTTGTCACATCT-3' (SEQ. ID. NO. 6); for 32 cycles with an annealing temperature of 62°C, generating a 589-bp fragment. Finally, a third aliquot of the same reverse-transcription reaction was amplified with the following primers for the constitutively expressed gene GAPDH: sense, 5'-CTCTAAGGCTGTGGGCAAGGTCAT-3' (SEQ. ID. NO. 7), antisense, 5'-GAGATCCACCACCCTGTTGCTGTA-3' (SEQ. ID. NO. 8). This reaction was carried out for 25 cycles with an annealing temperature of 58°C. In all cases, 10 µL of a 50-µL reaction were run on a 1.5% agarose gels.

**AMENDMENTS TO THE SEQUENCE LISTING**

The attached Sequence Listing conforms the Sequence Listing to standard United States patent practice. These sheets replace the original Sequence Listing.

Attachments: Sequence Listing and corresponding computer readable form (CRF).